

FIG. 1

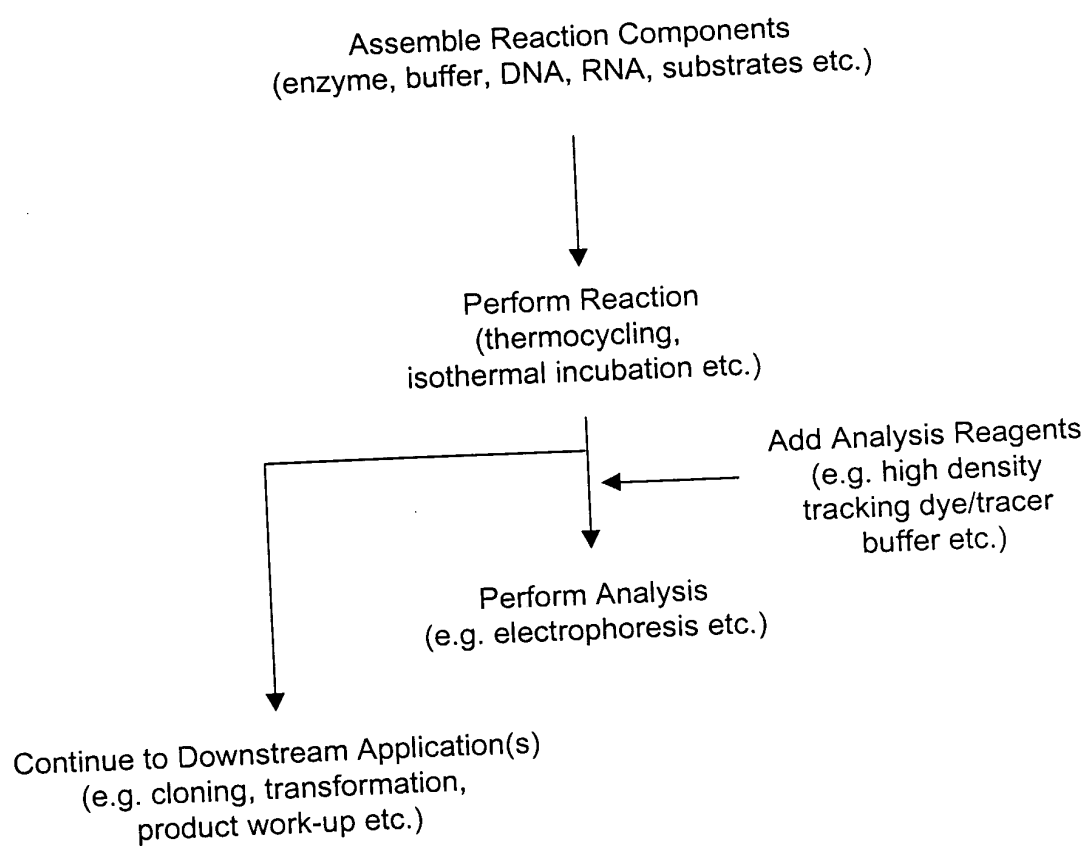


FIG. 2

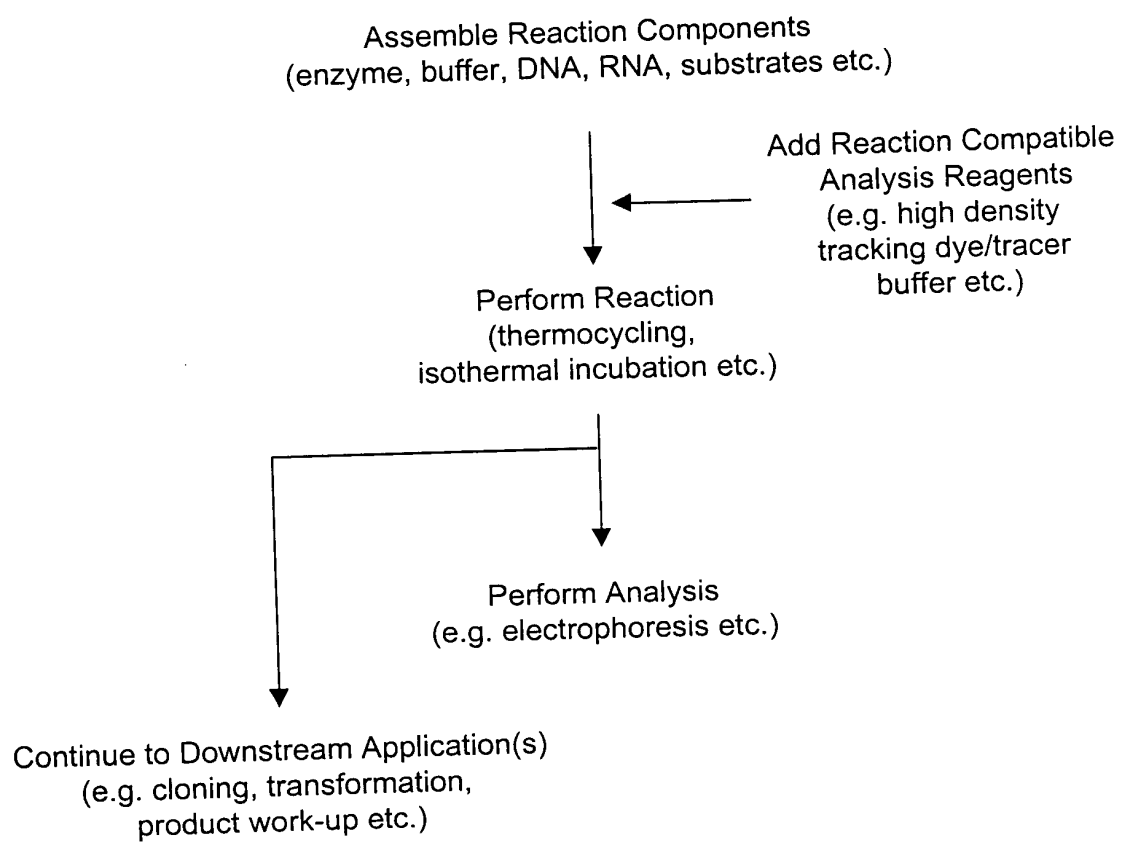


FIG. 3

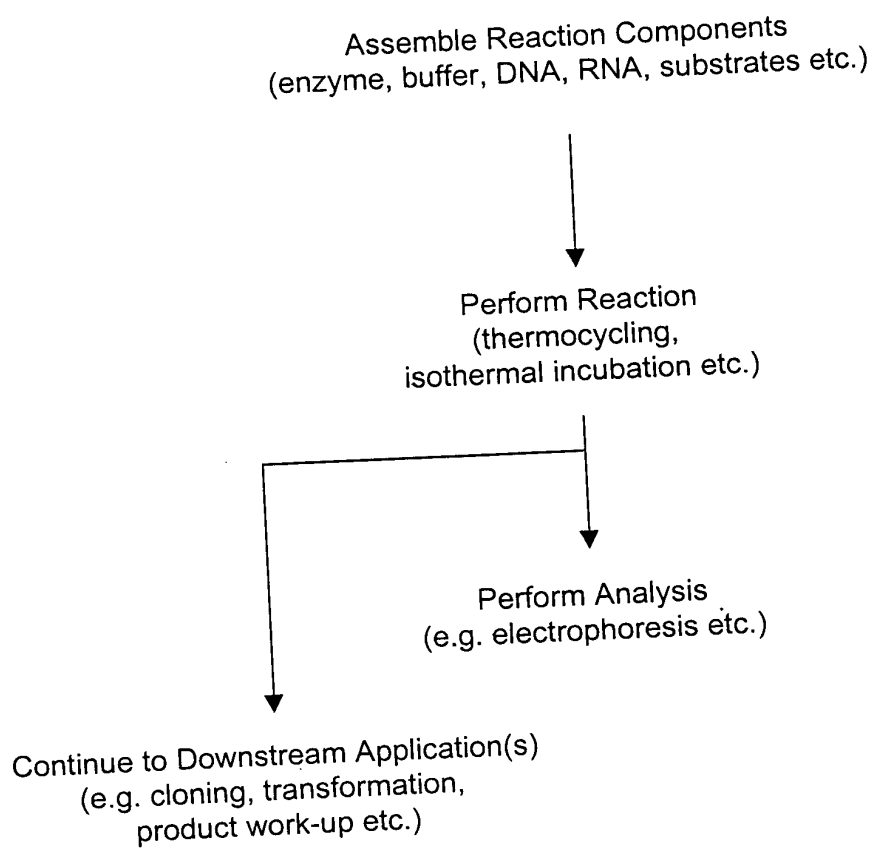


FIG. 4

Physical characteristics (e.g. high density to enable gel loading, colored to visualize gel loading, anionic chromophore to track electrophoresis progress, color (if desired)).



Assemble collection of reagents for screening (e.g. collect a large sampling of anionic dyes).



Screen molecules for compatibility. The screening might be best carried out by prioritizing the desired properties from least to most laborious (e.g. see red Taq Scheme 5).



Formulate reagent, characterize reaction products (qualitative and quantitative). Optimize other reaction components for perturbation (if necessary). Final characterization (reaction product quality and quantity) and limitations (e.g. incompatible with specific downstream applications)

FIG. 5

Physical characteristics.

High density to enable gel loading- formulate enzyme dilute enough so that enough glycerol will be contained in a 2.5 unit per 50 microliter reaction.

Colored to visualize gel loading and act as a tracking dye- red anionic water and ethanol soluble dyes were sought.



Assemble collection of reagents for screening - 40 anionic "red" (lambda max =450-550) dyes were selected as candidates.



Screen molecules for compatibility-summarized in Figure 1 . The dyes were scrutinized in the order:

1. Color (too yellow or purple thrown out)
2. Ethanol precipitation (colored DNA pellets thrown out)
3. Chaotropic salt/silica DNA purification (colored product thrown out)
4. PCR toxicity (Figure 2, low or no yield thrown out).
5. Ligase toxicity (Figure 3, low or no yield thrown out).
6. Transformation toxicity (Figure 4, low or no yield thrown out).
7. Remaining dyes more or less equivalent, submit to marketing for color selection.

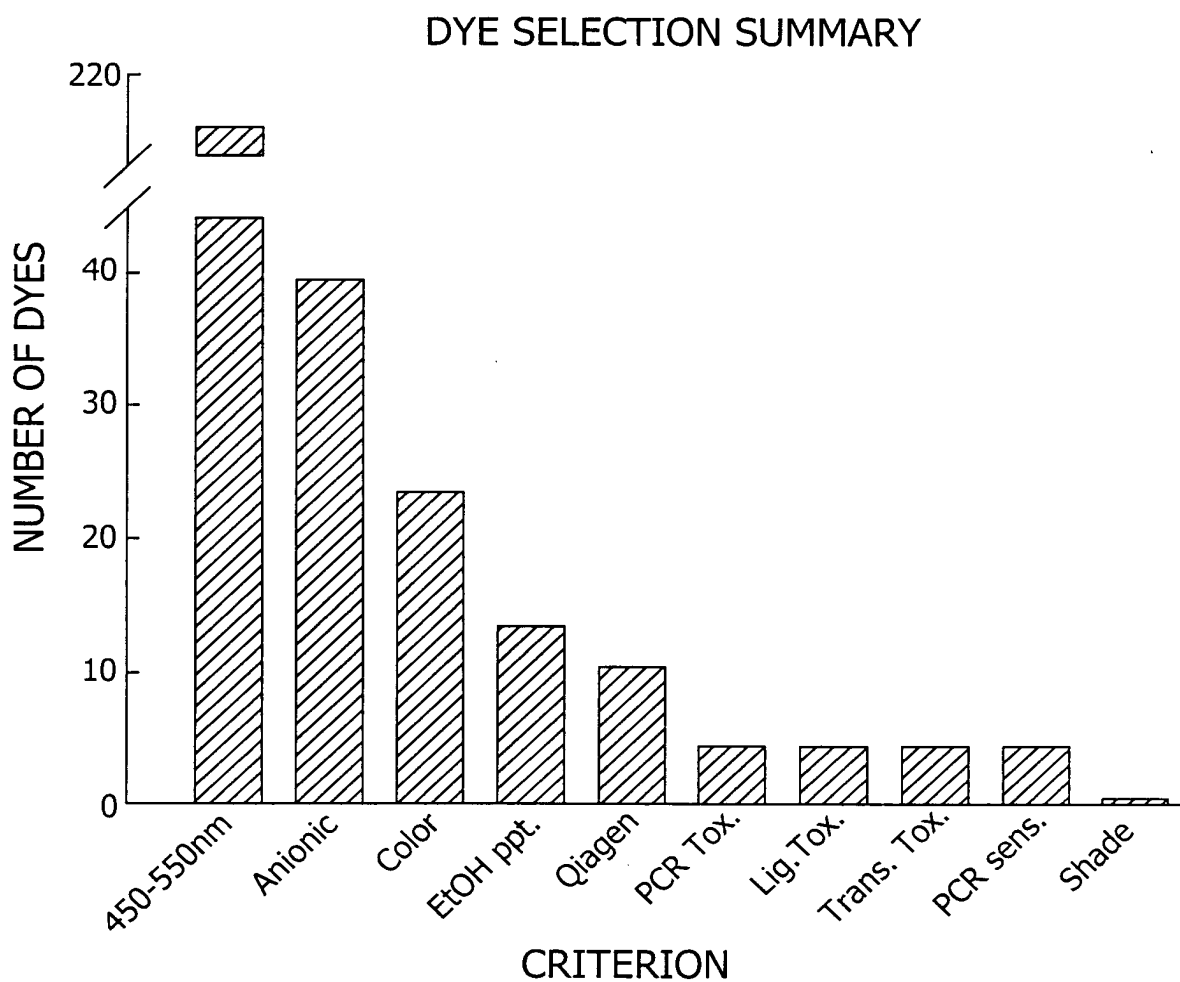


Formulate reagent- 80% acid red 1, 20% acid violet 5 (100%= absorbance of acid red 1 at lambda max + absorbance of acid violet 5 at lambda max.) to absorbance total =300 in Taq DNA polymerase at 1 unit per microliter in Taq storage buffer (20 mM Tris- HCl, pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween 20, 0.5% Igepal CA- 630, 50% glycerol)

Characterize reaction products-product yields low relative to absence of dye reactions (Figure 5). Dye purity (Figure 6) and counter ion identity (Figure 7) investigated for PCR toxicity/compatibility. Purified Mg acid red 1 and Mg Acid violet 5 found to be satisfactory.

Optimize other reaction components- Mg dyes contribute approximately 0.4 mM "free" Mg to PCR (Figure 8), 10X PCR buffer adjusted from 15 to 11 mM to accommodate Mg dye contribution. **Final characterization-** quality: gel (Figure 9), quantity (Figure 10). Limitations- will test with a panel of restriction enzymes, does not impact fluorescent sequencing.

FIG. 6



430-570 nm-visible absorption max.

Anionic-anionic dyes

Color-not too yellow/orange or purple

EtOH ppt. did not co-precipitate with DNA

Chaotropic salt/silica purification (Qiagen PCR columns)-isolated

DNA was colorless

PCR Tox.-little impact upon ³²P PCR product yield

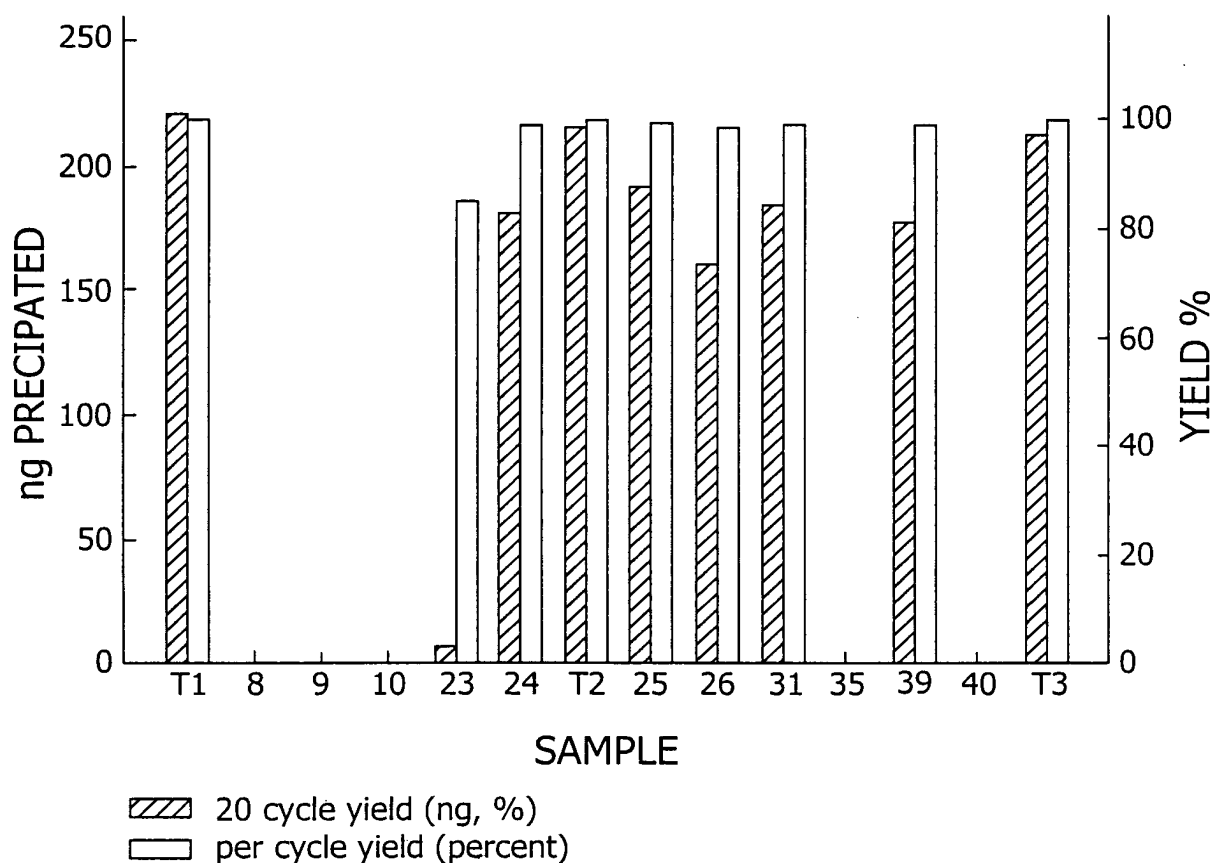
Lig. Tox.-little to no effect upon ligation of lambda PstI fragments

Trans. Tox.-no effect upon ligation/transformation of EcoRI-pUC19

PCR Sens.-amplification similar to no dye as a function of template concentration.

shade-marketing

FIG. 7



T1, T2 and T3= Taq controls (no dye) as in block and precipitation, numerals are dye number.
per cycle yield calculated assuming $y_{20} = y_1^{20}$ where y is the 20 cycle yield (measured) and y_1 is the per cycle yield.

Dye	$y(20\%)$	$y_1(\%)$
23	9.04	85.3
24	84.1	99.1
25	88.9	99.4
26	74.6	98.5
31	85.6	99.3
39	82.4	99.0

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FIG. 8

t_0 CTL 24 25 26 31 39

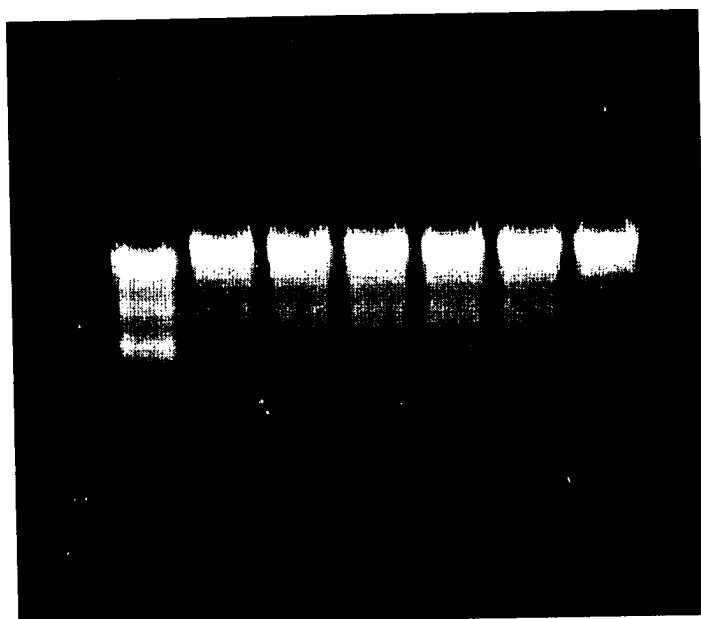
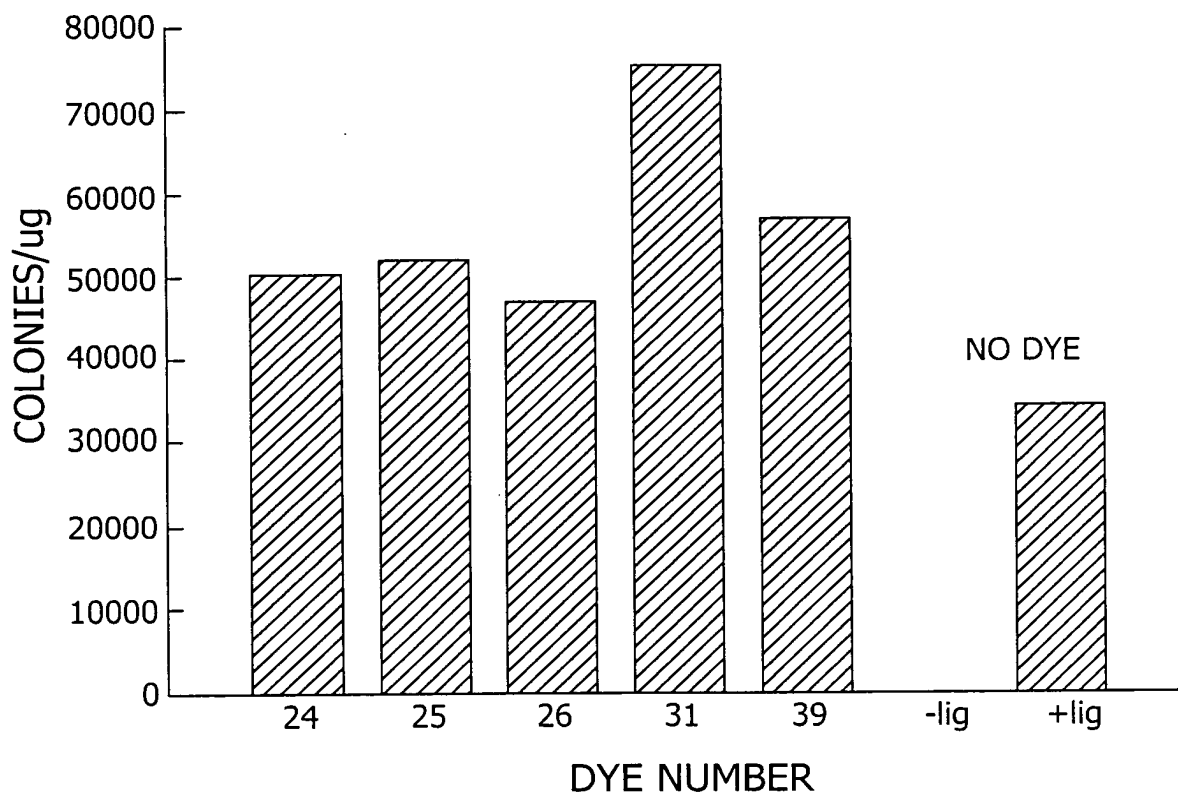


FIG. 9

TRANSFORMATION EFFICIENCY USING TAQ DYES



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FIG. 10



FIG. 11

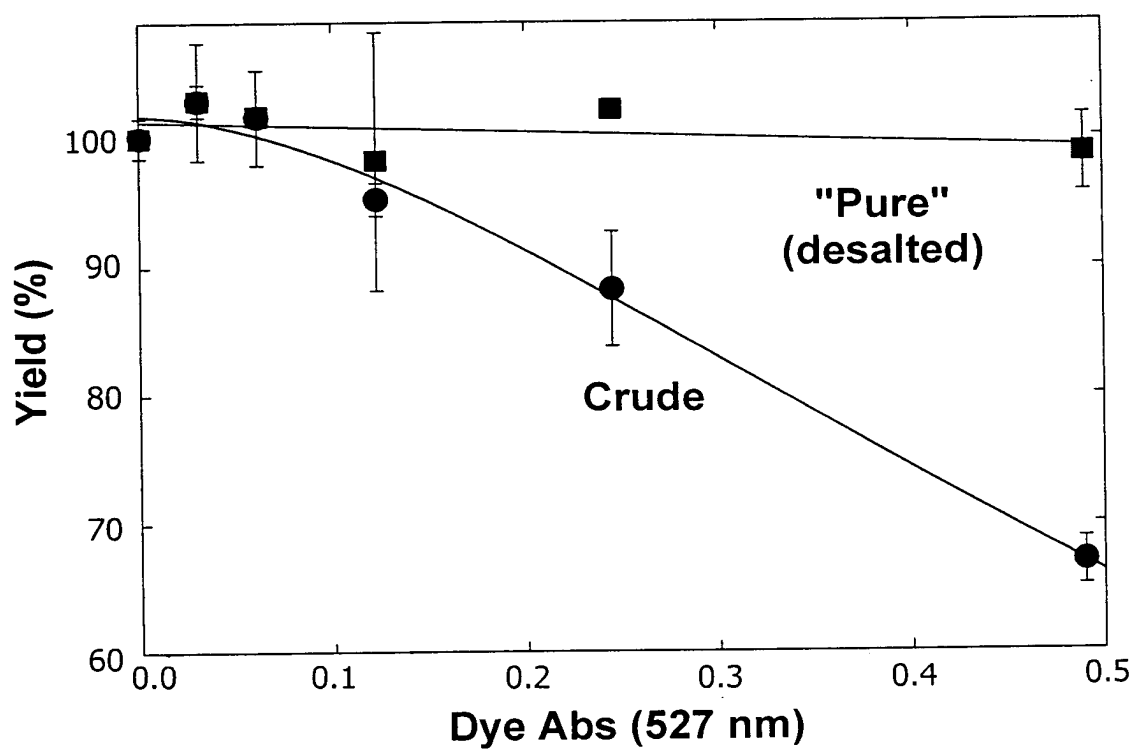


FIG. 12A

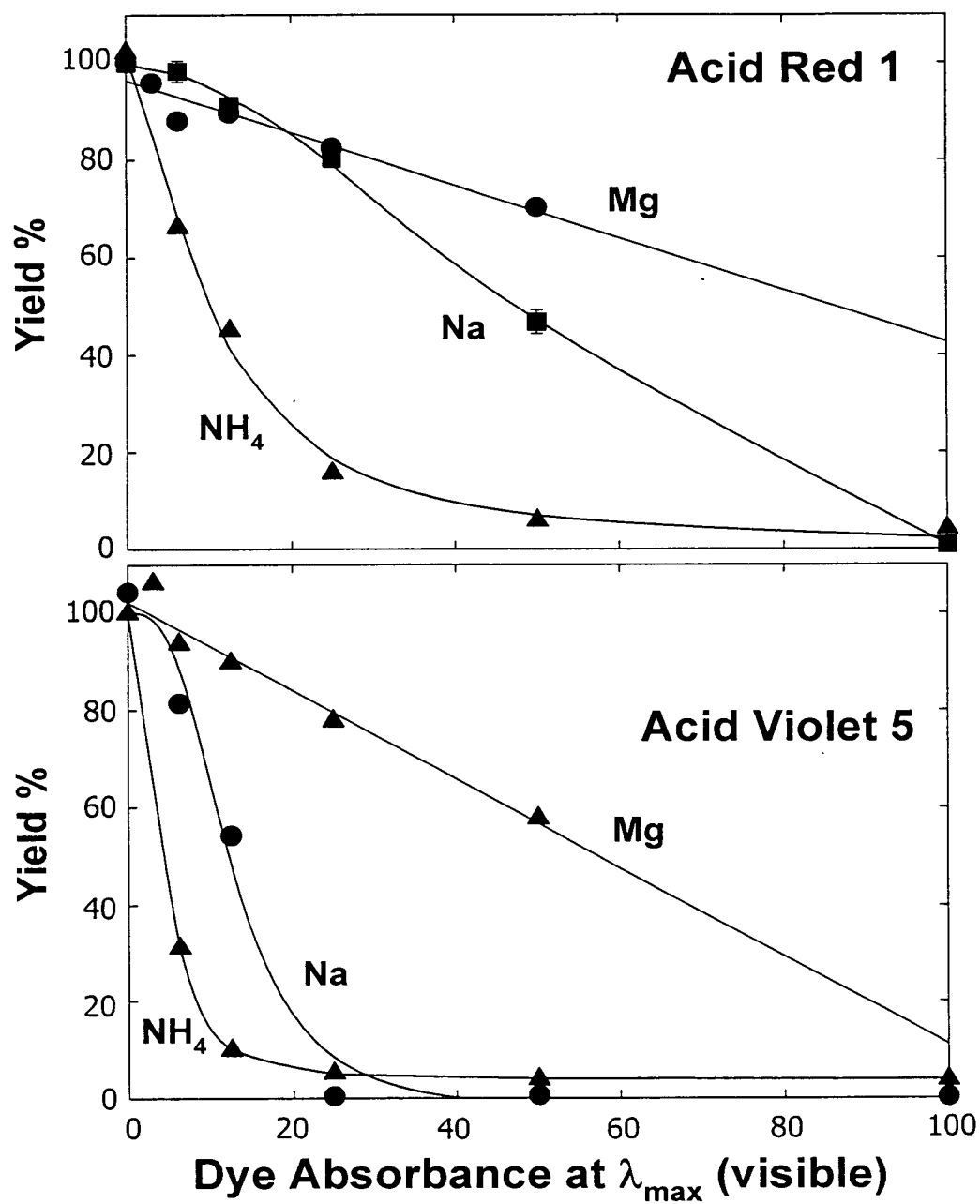


FIG. 12B

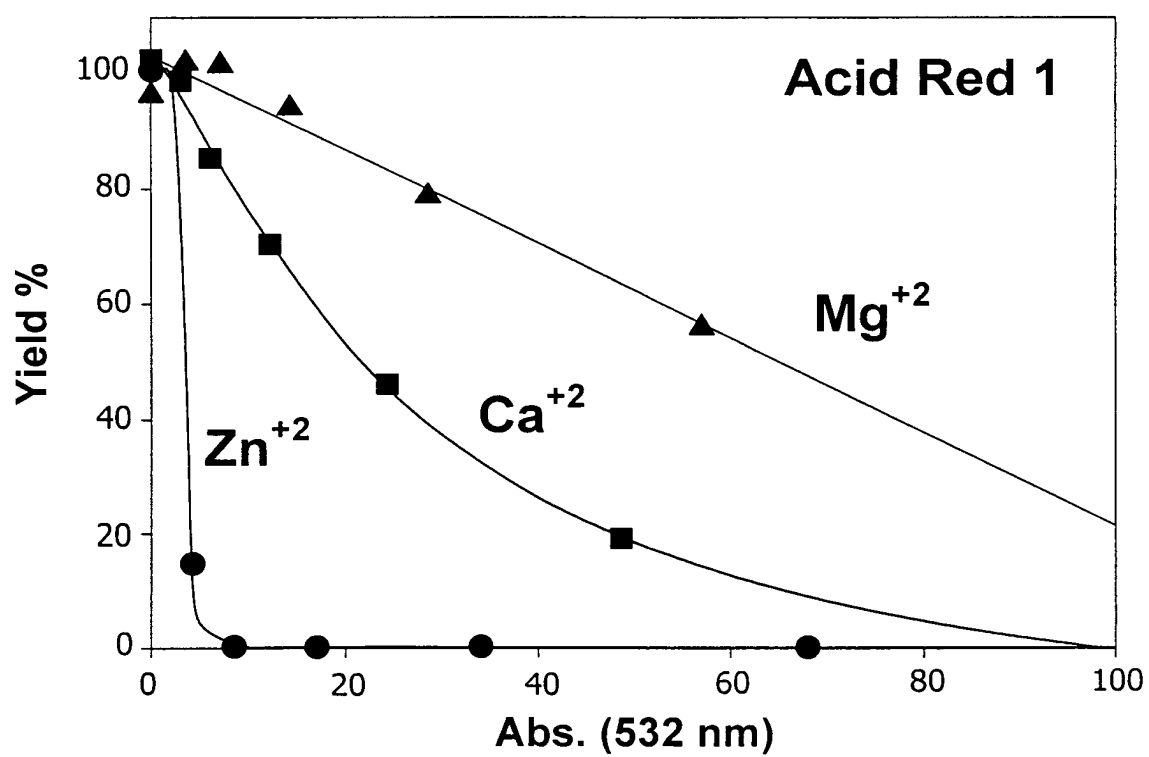
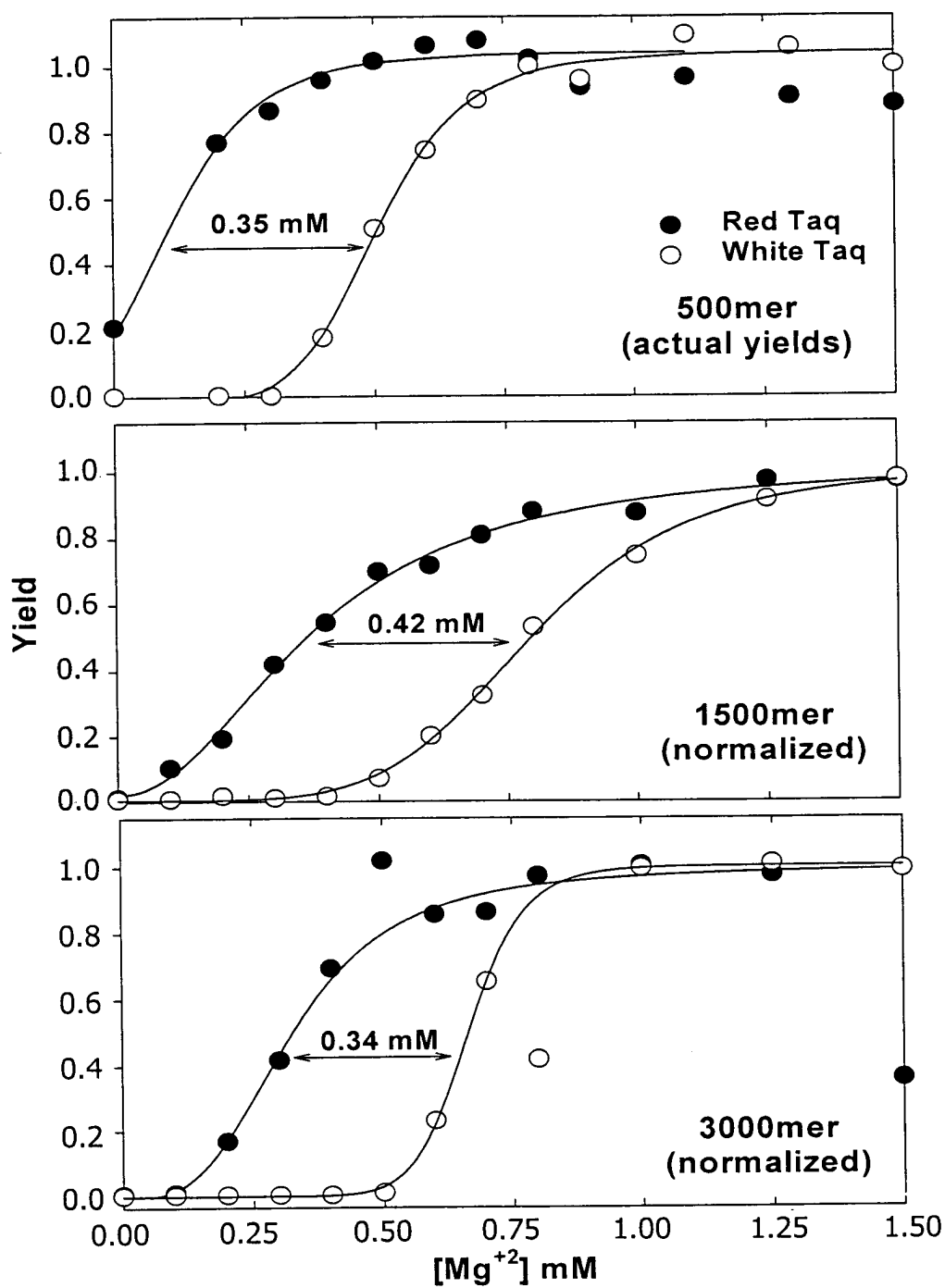


FIG. 13



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FIG. 14

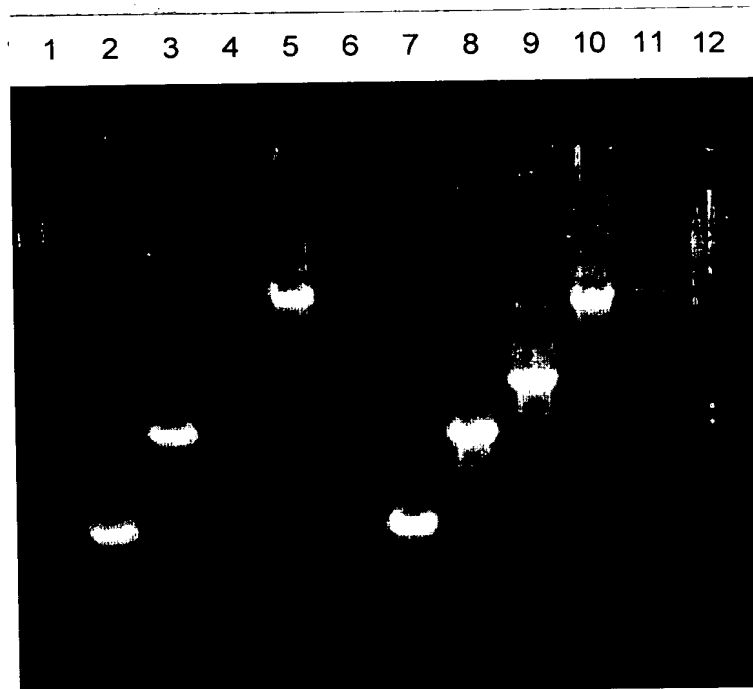


FIG. 15

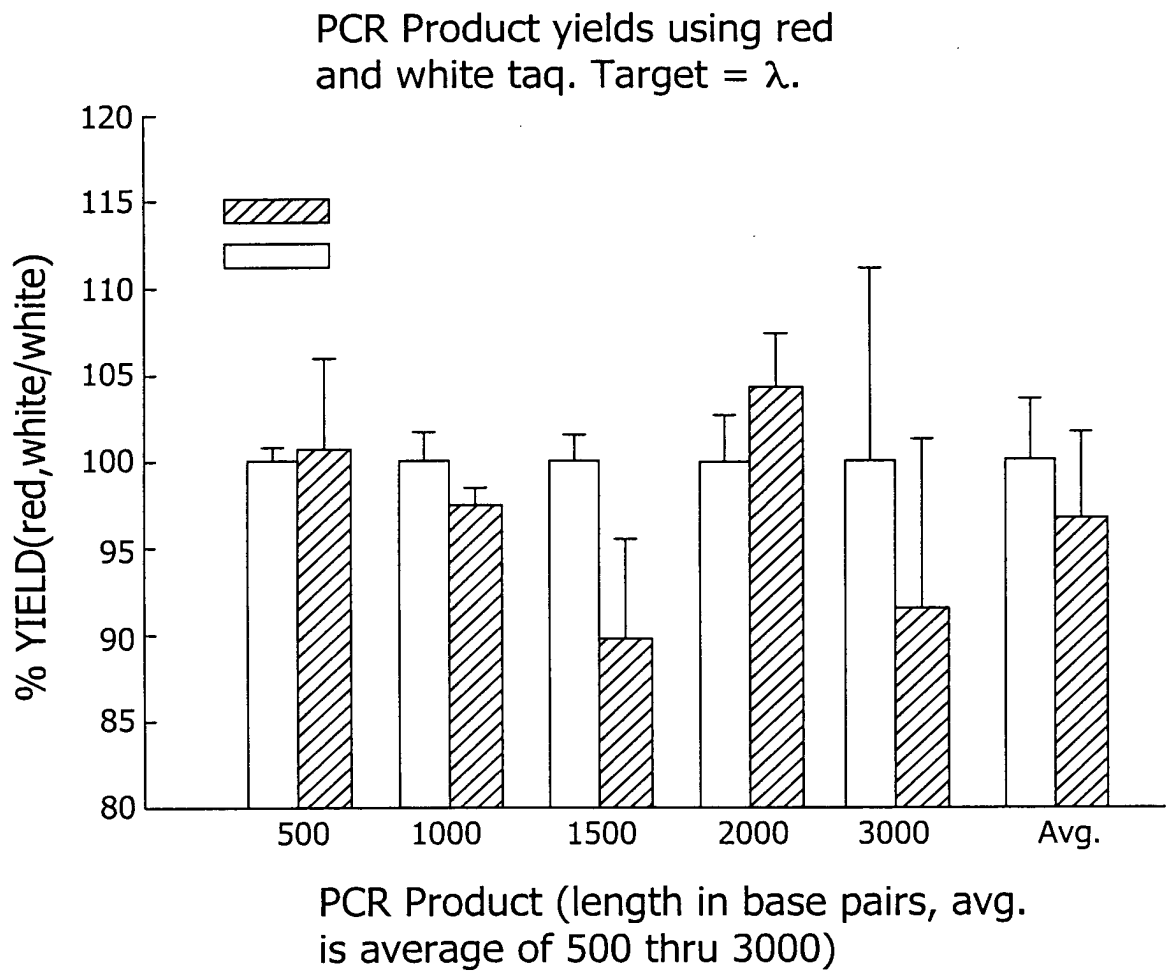
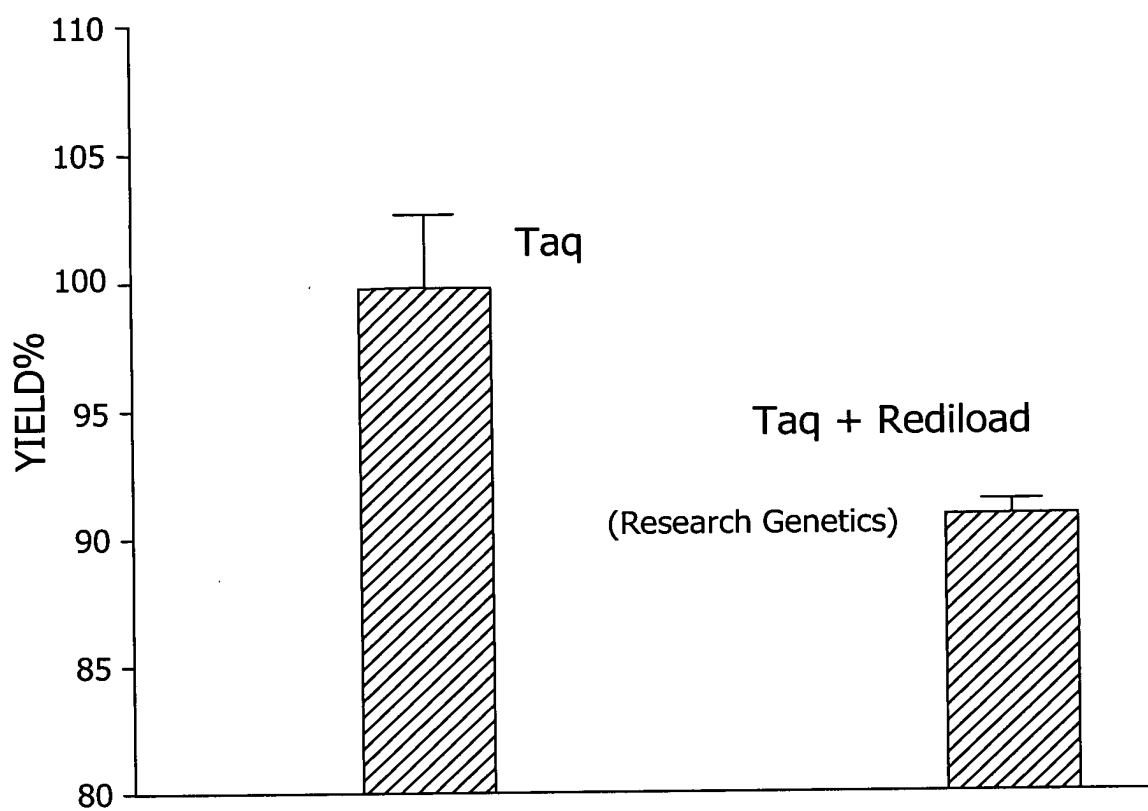


FIG. 16



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FIG. 17

